

**REMARKS**

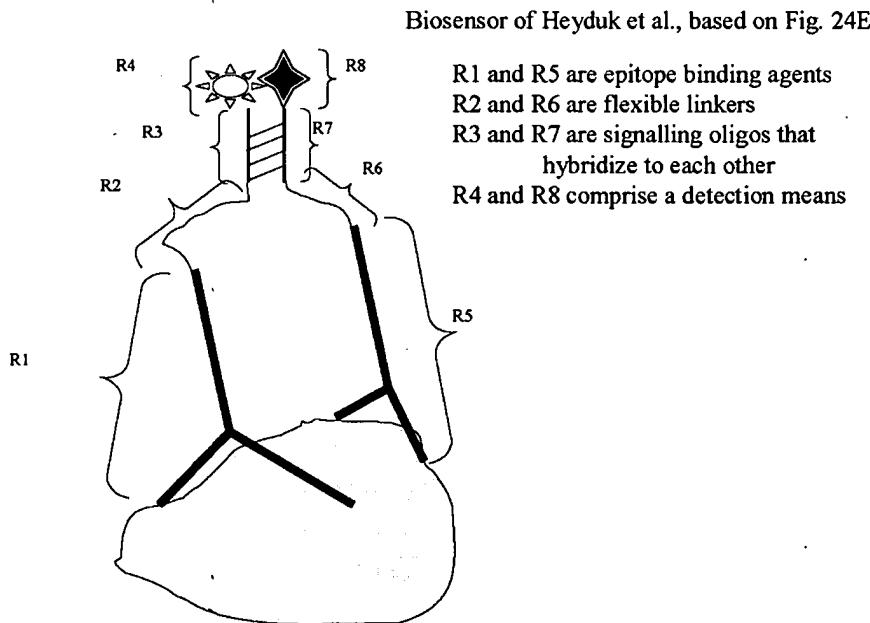
Claims 109-130 are pending. Claims 112-115, 117, and 128-130 have been withdrawn. Claim 118 is cancelled. Claims 109, 119, and 127 were previously amended. The text of claim 118 has been removed from the claim listing as requested by the Examiner. Hence, the objection to claim 118 is considered moot.

**I. §102 Rejection**

Reconsideration is requested of the rejection of claims 109-111, 116, 119-122, and 124-127 under 35 USC §102(b) in light of Baez et al. as evidenced by the HYTHER program.

Claim 109 encompasses a biosensor that comprises two nucleic acid constructs, represented by R1-R2-R3-R4 and R5-R6-R7-R8, as illustrated in Diagram 1A and Table A below.

Diagram 1A:



The Baez et al. application discloses a nucleic acid based reporter that may be used to detect an analyte. The Baez reporter uses two constructs, each comprising an antibody and a nucleic acid label as illustrated in Diagram 1B and Table A below.

Diagram 1B:

Biosensor of Baez et al, based on Fig. 1 and 3

According to the July 2 Office Action,  
A and D correspond to R1 and R5  
B and E correspond to R2 and R6  
C and F correspond to R3 and R7

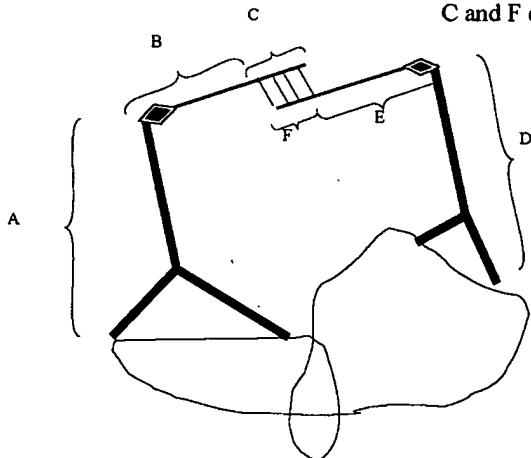


Table A presents an element by element comparison of the biosensor of claim 109 and the Baez reporter. Regardless of whether the July 2, 2008 Office Action or the March 12, 2009 Office Action interpretation of the Baez reporter is used, the Baez reporter does not disclose each element of claim 109. In particular, as discussed in more detail below, the Baez reporter does not disclose R2/R6 or R3/R7 as required by claim 109.

Table A

Claim 109	Baez disclosure, interpreted by the Office in the July 2, 2008 Office Action	Baez disclosure, interpreted by the Office in the March 12, 2009 Office Action
R1 / R5 epitope binding agent	A and D from Diagram 1B (i.e. antibody)	A and D from Diagram 1B (i.e. antibody)
R2 / R6 <u>non-nucleic acid</u> linker attaching R1 to R3	B and E from Diagram 1B (i.e. non- complementary <u>nucleic acid</u> attached to antibody via heterobifunctional linker)	diamond from Diagram 1B (i.e. heterobifunctional moiety)
R3 / R7 <u>complementary</u> nucleic acid sequences free energy between about 5.5- about 8.0 kcal/mol temperature about 21°C to 40°C salt at about 1mM to 100mM	C and F from Diagram 1B (i.e. complementary nucleic acid sequences) (See free energy discussion below)	B & C and E & F from Diagram 1B (i.e. <u>non-</u> <u>complementary</u> and complementary nucleic acid sequence, with a free energy above 8.0 kcal/mol under the conditions taught by Baez)
R4 / R8 Detection means	Detection means based on [00139] of the Baez application	Detection means based on [00139] of the Baez application

**(a) claim elements R2 and R6**

Baez does not disclose a non-nucleic acid linker. Per claim 109, R2 is a non-nucleic acid flexible linker attaching R1 to R3. R1 is an epitope binding agent (e.g. antibody), and R3 is a nucleic acid sequence complementary to R7. Stated another way, R2 connects the epitope binding agent to a complementary nucleic acid sequence. There is no non-nucleic acid portion of the Baez reporter that meets these requirements.

Consistent with the Applicants' premise that the cited reference only discloses nucleic acid linkers is the express teaching of Baez itself. Per the Baez application, the "nucleic acid label" of the Baez reporter comprises "three defined regions: a 5' sequence, the 3' sequence and a variable 'stuffer' sequence between the defined-terminal sequences."<sup>1</sup> The 5' end "has a chemically active group,"<sup>2</sup> "which allows it to be conjugated"<sup>3</sup> to the antibody via a cross-linking agent. Adjacent to the 5' end is the "stuffer" sequence, followed by the 3' sequence. Importantly, only the 3' end comprises the complementary sequence.

Hence, R3 and R7 of the Heyduk biosensor correlate to the 3' complementary end of the Baez reporter. This leaves the 5' nucleic acid sequence and the non-complementary "stuffer sequence," as the correlate to R2 (the flexible linker) of the Heyduk biosensor. But R2, per claim 109, cannot be nucleic acid. Consequently, the Baez reporter does not anticipate claim 109.

In the present Office Action, the Office contends that the heterobifunctional moiety (the diamond in Diagram 1B) is the equivalent of R2. Simply put, this is not correct. The heterobifunctional moiety in Baez connects the antibody to a nucleic acid sequence that IS NOT complementary, e.g. the 5' end of the nucleic acid label. (See Diagram 1B below and Figure 3 of the Baez application) Claim 109, in contrast, requires that R2 be attached to a complementary sequence (R3/R7). Hence, even if the heterobifunctional moiety did correspond to the non-nucleic acid linker, R2/R6, then the Office's

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<sup>1</sup> See paragraph [0145] of the Baez application.

<sup>2</sup> See paragraph [0147] of the Baez application.

<sup>3</sup> *Id.*

anticipation rejection would still fall short because there is no disclosure of R3/R7.

Importantly, the Applicants note that the Office's interpretation of what constitutes the Baez linker has remarkably changed since the last Office Action, where the Office agreed that the 5' end and the "stuffer" sequence correlated to the R2/R6 linker. In the July 2 Office Action, the Office stated:

"Baez et al further teaches that T66 and T68 comprise seven base pair complementary region at the 3' end.... The nucleic acid region comprising the complementary region in 'A' and 'A1' are the 'R3' and 'R7' of the instant claim. The linker comprising nucleic acid region not complementary to the nucleic acids in 'A' and 'A1' are the flexible linker R2...."<sup>4</sup>

Consistent with the Office's previous position, a skilled artisan empowered with the disclosure of Baez would not recognize the heterobifunctional moiety as a "linker." Instead, a skilled artisan would recognize that the heterobifunctional moiety is merely serving as the point of attachment between the antibody and the 5' end of the nucleic acid sequence. Such points of attachment are commonly used, for example, to join a protein sequence with a nucleic acid sequence. Without this point of attachment, there would be no efficient way of attaching the antibody to the 5' end of the Baez nucleic acid label. In this vein, the Applicants' respectfully submit that the heterobifunctional moiety of Baez does not correlate to the linker, R2/R6, of claim 109.

Assuming, for the sake of argument, that the Office's current position is correct, (i.e. Baez's heterobifunctional moiety corresponds to the linker of claim 109), the Baez reporter still does not anticipate claim 109. Under the Office's current position (i.e. that R2/R6 correlates to the heterobifunctional moiety in the Baez reporter), then the entire nucleic acid sequence of the Baez nucleic acid label must necessarily correlate with R3 and R7 to meet the requirements of

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<sup>4</sup> Office Action mailed July 2, 2008 at page 5.

claim 109.<sup>5</sup> (See Table A) Besides being outside the scope of claim 109 because the entire sequence is not complementary, as discussed above, the entire nucleic acid label does NOT have a free energy from about 5.5 kcal/mol to about 8.0 kcal/mol within the salt and temperature ranges of claim 109. For instance, see Table B, which details the free energies of the entire nucleic acid label of Baez (as calculated by the Hyther program)<sup>6</sup> under the hybridization conditions detailed in Baez (i.e. 25°C and 50mM NaCl)<sup>7</sup>, and under the high and low temperatures (about 21°C to about 40°C) of claim 109 along with a range of salt concentrations claimed (10mM to 100mM)<sup>8</sup>. Hence, even under the Office's current position, the Baez reporter does not anticipate claim 109.

Table B

Temperature (°C)	Salt	Free Energy
25	20mM	31.33
25	60mM	26.98
21	100mM	22.96
40	100mM	32.47
21	10mM	31.95
40	10mM	42.04

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<sup>5</sup> Claim 109 requires that R2/R6 attaches R1 to R3. Hence, if the heterobifunctional linker is characterized as R2 by the Office, R3 must necessarily correlate to the entire nucleic acid label of the Baez reporter. Any other interpretation would destroy the limitation, in claim 109, that R2 attaches R1 to R3.

<sup>6</sup> For a summary of the HYTHER calculation conditions, see Appendix 1.

<sup>7</sup> See paragraph [0131] of the Baez application. The annealing temperature was derived from the table following paragraph [0131] and Examples 1 and 2.

<sup>8</sup> Claim 109 is restricted to about 1mM to about 100mM. The HYTHER program, however, will not calculate free energy for salt conditions below 10mM. As a result, 10 mM is used in the calculations herein to represent the lower side of the salt concentration range.

"Anticipation under § 102 can be found only when the reference discloses exactly what is claimed . . ." Titanium Metals Corp. v. Banner, 778 F.2d 775, 780 (Fed. Cir. 1985) (citing D. Chisum, Patents § 3.02). "Because the hallmark of anticipation is prior invention, the prior art reference – in order to anticipate under 35 U.S.C. § 102 – must not only disclose all elements of the claim within the four corners of the document, but must also disclose those elements arranged as in the claim." Net Moneyin, Inc. v. Verisign, Inc., 545 F.3d 1359, 1369 (Fed. Cir. 2008) (citing Connell v. Sears, Roebuck & Co., 722 F.2d 1542, 1548 (Fed. Cir. 1983)). Baez does not disclose the R2/R6 and R3/R7 elements arranged as required by claim 109 of the present application. Consequently, Baez does not anticipate claim 109.

***(b) claim elements R3 and R7***

Baez does not disclose the free energy range required by claim 109. Claim 109 requires that R3 and R7 are complementary nucleic acid sequences that have a free energy range between about 5.5 kcal/mol and about 8.0 kcal/mol at a salt concentration from about 1mM to about 100mM and a temperature between about 21°C and 40°C. Baez discloses a complementary nucleic acid sequence of CGCCCGA. Using the hybridization conditions taught in Baez (25°C and KCl between 20-60mM)<sup>9</sup>, the Baez sequence does not have a free energy range between about 5.5 kcal/mol and about 8.0 kcal/mol, as required by Claim 109. While Baez may disclose a complementary sequence, the reference does not disclose use of this sequence under salt and temperature conditions that result in the free energy required by claim 109.

The Office incorrectly asserts that the Baez sequence has a free energy of 6.05 kcal/mol at 37°C and 50mM salt.<sup>10</sup> This, however, is the wrong temperature to evaluate the Baez sequence. These conditions (37°C, 50 mM

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<sup>9</sup> See paragraph [0131] of the Baez application.

<sup>10</sup> It is noted that the 6.05 kcal/mol value asserted by the Office was actually obtained using the sequence CGCCGA not CGCCCGA, according to the HYTHER conditions appended to the March 12, 2009 Office Action.

salt) may be within the purview of claim 109, but this is irrelevant in the determination of whether Baez discloses the claim element for free energy. What is important is not only the sequence taught by Baez, but also the conditions under which Baez utilizes this sequence. The Baez application explicitly states:

"[a] 7 to 10 bp overlap of the 3' ends of the labels, which has an approximate  $T_m$  of 25°C, was used to avoid formation of the duplex at 37°C. (the incubation temperature used for antibody-analyte binding).... However, after the analyte-reporter complex is formed and the temperature reduced to 25°C, the 3' overlap is allowed to form."<sup>11</sup>

Hence, the temperature for evaluating the free energy of the Baez sequence is 25°C, not 37°C. The free energy of the Baez sequence, at 25°C and a salt concentration of 50mM is 9.25 kcal/mol.<sup>12</sup> This is greater than the about 8.0 kcal/mol limitation of claim 109. Furthermore, the free energy of the Baez sequence, at 25°C over the range of salt conditions detailed in the Baez application (i.e. 20mM to 60mM)<sup>13</sup>, is still outside the range of claim 109, as detailed in Table C. Hence, the Baez reporter comprising the sequence CGCCCGA disclosed in the Baez application can not anticipate claim 109.

Table C

Temperature (°C)	Salt	Free Energy
25	20mM	8.65
25	60mM	9.37
25	50mM	9.25

Similar to claim 109, claims 110, 111, 116, 119 –122, and 124 -126 each depend from claim 109, and therefore necessarily incorporate each limitation of

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<sup>11</sup> See Baez application at paragraph [0184].

<sup>12</sup> See Appendix 1 for the calculation conditions.

<sup>13</sup> See paragraph [0131] of the Baez application

claim 109. Consequently, the Baez application cannot anticipate claims 110, 111, 116, 119 –122, and 124 -126 for the same reasons as detailed above with respect to claim 109.

Claim 119 is directed to the molecular biosensor of claim 109, wherein R2 forms a bond with each of R1 and R3 and R6 forms a bond with each of R5 and R7, wherein the free energy of the formed bonds is from about 12.0 kcal/mol to about 16.5 kcal/mol. The Office asserts that the "free energy of the formed bond is interpreted broadly as an obvious variant of the molecular sensor taught by Baez et al."<sup>14</sup> Applicants are not certain what the Office means by the phrase "obvious variant." To anticipate a claimed invention, a reference must disclose each element of the claimed invention. Baez et al does not disclose a free energy value for the bond between R1/R5 and R2/R6 and R2/R6 and R3/R7. Hence, Baez cannot anticipate claim 119 of the present invention.

Claim 127, analogous to claim 109, requires that R2 and R6 are not comprised of nucleic acid, and that R3 and R7 have a free energy of association between about 5.5 kcal/mol and 8.0 kcal/mol. The Baez application, which only discloses linkers of nucleic acids and does not disclose a complementary nucleic acid region with a free energy between about 5.5 kcal/mol and about 8.0 kcal/mol, does not, therefore, anticipate claim 127 for the same reasons detailed above with respect to claim 109.

Consequently, Applicant requests withdrawal of the rejection of claims 109-111, 116, 118-122 and 124-127 under §102b in view of Baez et al.

## II. §103 Rejection

Reconsideration is requested of the rejection of claims 109 and 123 under 35 USC §103(a) in view of Baez et al, as evidenced by the HYTHER program and Zaplinsky.

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<sup>14</sup> Office Action mailed March 12, 2009, at page 7.

As discussed above, claim 109 encompasses a biosensor comprising R1-R2-R3-R4 and R5-R6-R7-R8. None of the references cited by the Office disclose a biosensor with these elements in this arrangement.

Three criteria must be present to establish a *prima facie* case of obviousness.<sup>15</sup> First, the prior art reference must teach or suggest all the claim limitations. Second, there must be some suggestion or motivation in the knowledge generally available to one of ordinary skill in the art to modify the reference. Third, there must be a reasonable expectation of success.<sup>16</sup> Not one of these three criteria is satisfied by the combination of the Baez application, the HYTHER program, and the Zalipsky reference.

As discussed above, the Baez application discloses a reporter, but the Baez reporter does not disclose non-nucleic acid linkers, and does not teach a complementary nucleic acid sequence with a free energy of association between about 5.5 kcal/mol and 8.0 kcal/mol under the conditions of claim 109, as discussed above.

As stated by the Office, the HYTHER program was cited solely for the purpose of showing how to calculate free energy of association.

The Zalipsky reference discloses polyethyleneglycol (PEG) as a conjugate for biologically active molecules. By attaching PEG to the biologically active molecule, the molecule is stabilized. The Zalipsky reference does not disclose using PEG as a linker between an epitope binding agent and a complementary nucleic acid in a biosensor. Stated another way, the Zalipsky reference does not disclose attaching a chain of PEG to an epitope binding agent to connect the agent to a complementary nucleic acid. Nor does it disclose a biosensor with complementary nucleic acid sequences that have a free energy of association between about 5.5 kcal/mol and 8.0 kcal/mol.

In summary, not one of the cited references, whether taken together or in combination, disclose a biosensor with non-nucleic acid linkers.

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<sup>15</sup> MPEP §2143

<sup>16</sup> *Id.*

Moreover, there was no motivation in the art to modify the teachings of either the Baez reference or the Zalipsky reference. In particular, there was no motivation to alter the Baez reference to comprise a non-nucleic acid linker. To the contrary, the Baez application focuses on the benefits of using the nucleic acid labels to amplify the reporter signal via PCR amplification. Hence, the Baez application actually teaches away from using a non-nucleic acid linker, which could not be amplified with PCR.

Additionally, there was no motivation to alter the Zalipsky reference to create a biosensor with a PEG-based linker. Zalipsky was concerned with the stability of the biologically active molecule. In contrast, the present application utilizes PEG to tether an epitope binding agent to a complementary nucleic acid sequence. Zalipsky provides no disclosure or motivation to use PEG as anything but a stabilizer, but the stability of a molecule is not a factor in the biosensor of claim 109. Therefore, there would have been no motivation to use the Zalipsky reference to modify the Baez reporter to arrive at claim 109. As such, the Office has failed to put form a *prima facie* case of obviousness against claims 109 and 123.

Hence, Applicants respectfully submit that claim 109 is patentable over the cited art. Similarly, claim 123 depends from claim 109, and therefore, is patentable for the same reasons as detailed above with respect to claim 109. Applicants therefore request withdrawal of the rejection of claims 109 and 123 for obviousness under §103(a).

### **III. Double Patenting Rejections**

Claims 109-111, 116, and 119-127 are rejected for provisional nonstatutory obviousness-type double patenting in view of claims 1-11 of copending application no. 11/836,339 and claims 1-8 of copending application no. 11/836,333. Because the '339 and '333 applications have not issued as patents, as the Office correctly notes, the double patenting rejection is a provisional rejection. As such, the applicants will address the substantive merit of the double

**PATENT**  
Atty. Docket No. 048483-102364  
Via EFS Web

patenting rejection if and when either of the '339 or '332 applications issue as patents.

**CONCLUSION**

In light of the foregoing, applicants request entry of the claim amendments, withdrawal of the claim rejections, and solicit an allowance of the claims. The Examiner is invited to contact the undersigned attorney should any issues remain unresolved.

Respectfully submitted,  
Polsinelli Shalton Flanigan Suelthaus PC

Date: May 12, 2009

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# Appendix 1

Welcome 209.135.145.206 !



Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

Menu

Lab Homepage

### Duplex sequence

5'-CGGGGGAGACGAAACTGCTAACTTATATTCCCTCCTACTTGCATCACCAACCCATTCCGCCGA-  
 3'  
 3'-GCGGGCTCTAGTATTAAATTGCGTATCTAACCTTCGTCTCTCTCAGAACAAAGCCGTAGGAGCG-  
 5'

### Experimental conditions

Hybridization type = DNA/DNA  
 [Top strand] = 2.00e-004 mol/L  
 [Bottom strand] = 2.00e-004 mol/L  
 Hybridization temperature = 25.0 °C  
 [Monovalent cation] = 0.06 mol/L  
 [Mg<sup>2+</sup>] = 0.00 mol/L

### Corrections

No corrections for microchips  
 and single strand folding!

### Thermodynamic predictions

In 0.0600 M NaCl and 0.0000 M MgCl<sub>2</sub>:  
 $\Delta H^\circ = -124.30$  kcal/mol  
 $\Delta S^\circ = -507.41$  eu  
 $\Delta G^\circ_{25.0} = 26.98$  kcal/mol  
 $T_M = -36.7$  °C

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HYTHER<sup>TM</sup> server - Nicolas Peyret, Pirro Saro and John SantaLucia, Jr.

Department of Chemistry, Wayne State University

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Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

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Lab Homepage

## Duplex sequence

5'-  
 CGGGGGAGACGAAACTGCTAACTTATTCCTTCCTACTTGCATCACCAACCCATTCCGCCGA-  
 3'  
 3'-  
 GCGGGCTCTAGTATTAATTGCGTATCTAACCTTCGTCTCTCAGAACAAACAAGCGGTAGGAGCG-  
 5'

## Experimental conditions

Hybridization type = DNA/DNA

[Top strand] = 2.00e-004 mol/L

[Bottom strand] = 2.00e-004 mol/L

Hybridization temperature = 25.0 °C

[Monovalent cation] = 0.02 mol/L

[Mg<sup>2+</sup>] = 0.00 mol/L

## Corrections

No corrections for microchips  
and single strand folding!

## Thermodynamic predictions

In 0.0200 M NaCl and 0.0000 M MgCl<sub>2</sub>:

ΔH° = -124.30 kcal/mol

ΔS° = -521.97 eu

ΔG°<sub>25.0</sub> = 31.33 kcal/molT<sub>M</sub> = -43.1 °CThere have been **365124** submissions from external users on this server since September 1999.

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Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

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## Duplex sequence

5'-  
 CGGGGGAGACGAAACTGCTAACTTATATTCCCTCCTACTTGCATCACCAACCCATTCCGCCGA-  
 3'  
 3'-  
 GCGGGCTCTAGTATTAATTGCGTATCTAACCTTCGTCTCTCAGAACAAACAAAGCGGTAGGAGCG-  
 5'

## Experimental conditions

Hybridization type = DNA/DNA

[Top strand] = 2.00e-004 mol/L

[Bottom strand] = 2.00e-004 mol/L

Hybridization temperature = 40.0 °C

[Monovalent cation] = 0.10 mol/L

[Mg<sup>2+</sup>] = 0.00 mol/L

## Corrections

No corrections for microchips  
and single strand folding!

## Thermodynamic predictions

In 0.1000 M NaCl and 0.0000 M MgCl<sub>2</sub>: $\Delta H^\circ = -124.30$  kcal/mol $\Delta S^\circ = -500.63$  eu $\Delta G^\circ_{40.0} = 32.47$  kcal/mol $T_M = -33.6$  °CThere have been **365121** submissions from external users on this server since September 1999.HYTHER<sup>TM</sup>server - Nicolas Peyret, Pirro Saro and John SantaLucia, Jr.

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Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

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## Duplex sequence

5'-  
 CGGGGGAGACGAAACTGCTAACTTATATTCCCTCCTACTTGCATCACCAACCCATTCCGCCGA-  
 3'  
 3'-  
 GCGGGCTCTAGTATTAATTGCGTATCTAACCTTCGTCTCTCTCAGAACACAAGCGGTAGGAGCG  
 5'

## Experimental conditions

Hybridization type = DNA/DNA  
 [Top strand] = 2.00e-004 mol/L  
 [Bottom strand] = 2.00e-004 mol/L  
 Hybridization temperature = 40.0 °C  
 [Monovalent cation] = 0.01 mol/L  
 [Mg<sup>2+</sup>] = 0.00 mol/L

## Corrections

No corrections for microchips  
 and single strand folding!

## Thermodynamic predictions

In 0.0100 M NaCl and 0.0000 M MgCl<sub>2</sub>:  
 $\Delta H^\circ = -124.30$  kcal/mol  
 $\Delta S^\circ = -531.17$  eu  
 $\Delta G^\circ_{40.0} = 42.04$  kcal/mol  
 $T_M = -46.9$  °C

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Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

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Duplex sequence

5'-  
CGGGGGAGACGAAACTGCTAACTTATATTCCCTCCTACTTGCATCACCAACCCATTCCGCCGA-  
3'  
3'-  
GCGGGCTCTAGTATTAATTGCGTATCTAACCTTCGTCTCTCAGAACAAAGCGGTAGGAGCG-  
5'

Experimental conditions

Hybridization type = DNA/DNA

[Top strand] = 2.00e-004 mol/L

[Bottom strand] = 2.00e-004 mol/L

Hybridization temperature = 21.0 °C

[Monovalent cation] = 0.10 mol/L

[Mg<sup>2+</sup>] = 0.00 mol/L

Corrections

No corrections for microchips  
and single strand folding!

Thermodynamic predictions

In 0.1000 M NaCl and 0.0000 M MgCl<sub>2</sub>:

$\Delta H^\circ = -124.30$  kcal/mol

$\Delta S^\circ = -500.63$  eu

$\Delta G^\circ_{21.0} = 22.96$  kcal/mol

$T_M = -33.6$  °C

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Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

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## Duplex sequence

5'  
 CGGGGGAGACGAAACTGCTAACTTATTCCTCCTACTTGCATCACCAACCCATTCCGCCGA-  
 3'  
 3'  
 GCGGGCTCTAGTATTAATTGCGTATCTAACCTTCGTCTCTCAGAACAAACAAGCGTAGGAGCG-  
 5'

## Experimental conditions

Hybridization type = DNA/DNA  
 [Top strand] = 2.00e-004 mol/L  
 [Bottom strand] = 2.00e-004 mol/L  
 Hybridization temperature = 21.0 °C  
 [Monovalent cation] = 0.01 mol/L  
 [Mg<sup>2+</sup>] = 0.00 mol/L

## Corrections

No corrections for microchips  
 and single strand folding!

## Thermodynamic predictions

In 0.0100 M NaCl and 0.0000 M MgCl<sub>2</sub>:  
 $\Delta H^\circ = -124.30$  kcal/mol  
 $\Delta S^\circ = -531.17$  eu  
 $\Delta G^\circ_{21.0} = 31.95$  kcal/mol  
 $T_M = -46.9$  °C

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Department of Chemistry, Wayne State University

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Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

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**Duplex sequence**

5'-CGCCCGA-3'  
3'-GCGGGCT-5'

**Experimental conditions**

Hybridization type = DNA/DNA

[Top strand] = 2.00e-004 mol/L

[Bottom strand] = 2.00e-004 mol/L

Hybridization temperature = 25.0 °C

[Monovalent cation] = 0.02 mol/L

[Mg<sup>2+</sup>] = 0.00 mol/L**Corrections**No corrections for microchips  
and single strand folding!**Thermodynamic predictions**In 0.0200 M NaCl and 0.0000 M MgCl<sub>2</sub>: $\Delta H^\circ = -52.80 \text{ kcal/mol}$  $\Delta S^\circ = -148.09 \text{ eu}$  $\Delta G^\circ_{25.0} = -8.65 \text{ kcal/mol}$  $T_M = 44.2 \text{ }^\circ\text{C}$ There have been **365129** submissions from external users on this server since September 1999.HYTHER<sup>TM</sup> server - Nicolas Peyret, Pirro Saro and John SantaLucia, Jr.  
Department of Chemistry, Wayne State University

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Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

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Duplex sequence

5'-CGCCCGA-3'  
3'-GCGGGCT-5'

Experimental conditions

Hybridization type = DNA/DNA

[Top strand] = 2.00e-004 mol/L

[Bottom strand] = 2.00e-004 mol/L

Hybridization temperature = 25.0 °C

[Monovalent cation] = 0.06 mol/L

[Mg<sup>2+</sup>] = 0.00 mol/L

Corrections

No corrections for microchips  
and single strand folding!

Thermodynamic predictions

In 0.0600 M NaCl and 0.0000 M MgCl<sub>2</sub>:

$\Delta H^0 = -52.80$  kcal/mol

$\Delta S^0 = -145.66$  eu

$\Delta G^0_{25.0} = -9.37$  kcal/mol

$T_M = 48.9$  °C

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Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

Menu
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## Duplex sequence

5'-CGCCCGA-3'  
3'-GCGGGCT-5'

## Experimental conditions

Hybridization type = DNA/DNA

[Top strand] = 2.00e-004 mol/L

[Bottom strand] = 2.00e-004 mol/L

Hybridization temperature = 25.0 °C

[Monovalent cation] = 0.05 mol/L

[Mg<sup>2+</sup>] = 0.00 mol/L

## Corrections

No corrections for microchips  
and single strand folding!

## Thermodynamic predictions

In 0.0500 M NaCl and 0.0000 M MgCl<sub>2</sub>: $\Delta H^\circ = -52.80$  kcal/mol $\Delta S^\circ = -146.06$  eu $\Delta G^\circ_{25.0} = -9.25$  kcal/mol $T_M = 48.1$  °CThere have been **365133** submissions from external users on this server since September 1999.

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Prediction of Nucleic Acid Hybridization Thermodynamics

Module 1

Menu

Lab Homepage

## Duplex sequence

5'-CGCCCGA-3'

3'-GCGGGCT-5'

## Experimental conditions

Hybridization type = DNA/DNA

[Top strand] = 2.00e-004 mol/L

[Bottom strand] = 2.00e-004 mol/L

Hybridization temperature = 37.0 °C

[Monovalent cation] = 0.05 mol/L

[Mg<sup>2+</sup>] = 0.00 mol/L

## Corrections

No corrections for microchips  
and single strand folding!

## Thermodynamic predictions

In 0.0500 M NaCl and 0.0000 M MgCl<sub>2</sub>: $\Delta H^\circ = -52.80$  kcal/mol $\Delta S^\circ = -146.07$  eu $\Delta G^\circ_{37.0} = -7.50$  kcal/mol $T_M = 48.1$  °CThere have been **365146** submissions from external users on this server since September 1999.HYTHER<sup>TM</sup> server - Nicolas Peyret, Pirro Saro and John SantaLucia, Jr.

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